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Page #6

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FILE 'USPAT' ENTERED AT 15:51:56 ON 29 DEC 1997

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  *                                                                 *
  *              W E L C O M E   T O   T H E                        *
  *         U. S.   P A T E N T   T E X T   F I L E                 *
  *                                                                 *
  * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

=> s polytetrafluoroethylene?
L1        25234 POLYTETRAFLUOROETHYLENE?

=> s l1 and implant?
          52487 IMPLANT?
L2         1420 L1 AND IMPLANT?

=> s l2 and gene therap?
          15178 GENE
          74969 THERAP?
            870 GENE THERAP?
                  (GENE(W)THERAP?)
L3           10 L2 AND GENE THERAP?

=> d l3,1-10,cit
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1. 5,658,565, Aug. 19, 1997, Inducible nitric oxide synthase gene for treatment of disease; Timothy R. Billiar, et al., 424/93.21, 93.1, 93.2; 435/172.3, 189, 191, 235.1, 320.1; 514/44; 536/23.1, 23.2, 23.5; 935/9, 22, 32, 60 :IMAGE AVAILABLE:

2. 5,626,561, May 6, 1997, **Implantable** containment apparatus for a therapeutical device and method for loading and reloading the device therein; Mark D. Butler, et al., 604/49, 93, 890.1 :IMAGE AVAILABLE:

3. 5,624,840, Apr. 29, 1997, Three-dimensional liver cell and tissue culture system; Brian A. Naughton, et al., 435/395; 424/423; 435/373, 399, 402 :IMAGE AVAILABLE:

4. 5,599,788, Feb. 4, 1997, Method for accelerating skin wound healing with H3 protein; Anthony F. Purchio, et al., 514/2; 424/278.1, 409; 514/12, 885, 886, 887, 944, 945, 946, 947 :IMAGE AVAILABLE:

5. 5,594,136, Jan. 14, 1997, Texaphyrin solid supports and devices; Jonathan L. Sessler, et al., 540/472; 424/9.322; 534/11, 14, 15, 16; 540/145, 474 :IMAGE AVAILABLE:

6. 5,516,681, May 14, 1996, Three-dimensional pancreatic cell and tissue culture system; Gail K. Naughton, et al., 435/353; 424/422, 484, 572; 435/1.1, 1.2, 29, 32, 284.1, 347, 373 :IMAGE AVAILABLE:

7. 5,510,254, Apr. 23, 1996, Three dimensional cell and tissue culture system; Brian A. Naughton, et al., 435/370, 284.1 :IMAGE AVAILABLE:

8. 5,424,208, Jun. 13, 1995, Method for isolating cells from tissue with a composition containing collagenase and chymopapin; Catherine T. Lee, et al., 435/268, 219, 243, 267, 381 :IMAGE AVAILABLE:

9. 5,422,261, Jun. 6, 1995, Composition containing collagenase and chymopapain for hydrolyzing connective tissue to isolate cells; Catherine

T. Lee, et al., 435/219; 424/94.2, 94.65, 94.67; 435/212 :IMAGE AVAILABLE:

10. 4,963,489, Oct. 16, 1990, Three-dimensional cell and tissue culture system; Gail K. Naughton, et al., 435/1.1; 424/529, 530, 534, 572, 574; 435/2, 347, 366, 398, 402 :IMAGE AVAILABLE:

=> d kwic,10

5599712

US PAT NO: 4,963,489 :IMAGE AVAILABLE: L3: 10 of 10

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SUMMARY:

BSUM(3)

The resulting cultures have a variety of applications ranging from transplantation or **implantation** in vivo, to screening cytotoxic compounds and pharmaceutical compounds in vitro, and to the production of biologically active molecules in. . .

SUMMARY:

BSUM(14)

The . . . can be grown in the three-dimensional culture system. The resulting cultures have a variety of applications ranging from transplantation or **implantation**, in vivo, of cells grown in the cultures, cytotoxicity testing and screening compounds in vitro, and the design of "bioreactors". . .

DETDESC:

DETD(3)

The . . . variety of applications. For example, for tissues such as skin, glands, etc. the three-dimensional culture itself may be transplanted or **implanted** into a living organism. Alternatively, for diffuse tissues such as bone marrow, the proliferating cells could be isolated from the. . .

DETDESC:

DETD(5)

Fetal . . . particular tissue, organ, or individual. For example, where the three-dimensional culture is to be used for purposes of transplantation or **implantation** in vivo, it may be preferable to obtain the stromal cells and elements from the individual who is to receive the transplant or **implant**. This approach might be especially advantageous where immunological rejection of the transplant and/or graft versus host disease is likely. Moreover,. . .

DETDESC:

DETD(17)

Where the three-dimensional culture is itself to be **implanted** in vivo, it may be preferable to use biodegradable matrices such as PGA, catgut suture material, or gelatin, for example.. . .

DETDESC:

DETD(23)

Again, where the cultured cells are to be used for transplantation or

implantation in vivo it is preferable to obtain the stromal cells from the patient's [redacted]ssues. The growth of cells in[redacted] .

DETDESC:

DETD(37)

The . . . of the invention can be used in a variety of applications. These include but are not limited to transplantation or **implantation** of either the cultured cells obtained from the matrix, or the cultured matrix itself in vivo; screening cytotoxic compounds, allergens,. . . of certain diseases; studying the mechanism by which drugs and/or growth factors operate; diagnosing and monitoring cancer in a patient; **gene therapy**; and the production of biologically active products, to name but a few.

DETDESC:

DETD(38)

For transplantation or **implantation** in vivo, either the cells obtained from the culture or the entire three-dimensional culture could be **implanted**, depending upon the type of tissue involved. For example, three-dimensional bone marrow cultures can be maintained in vitro for long. . .

DETDESC:

DETD(42)

The . . . culture system of the invention may afford a vehicle for introducing genes and gene products in vivo for use in **gene therapies**. For example, using recombinant DNA techniques, a gene for which a patient is deficient could be placed under the control. . . and then clonally expanded in the three-dimensional culture system. The three-dimensional culture which expresses the active gene product, could be **implanted** into an individual who is deficient for that product.

DETDESC:

DETD(43)

The use of the three-dimensional culture in **gene therapy** has a number of advantages. Firstly, since the culture comprises eukaryotic cells, the gene product will be properly expressed and processed in culture to form an active product. Secondly, **gene therapy** techniques are useful only if the number of transfected cells can be substantially enhanced to be of clinical value, relevance,. . .

DETDESC:

DETD(196)

Twenty . . . 6 mm punches were made with a disposable Baker's punch biospy needle, and sub-cuticular suturing was used to hold the **implanted** meshes in place. The rats were closely examined until 12 hours post surgery and then monitored every 24 hours.

DETDESC:

DETD(197)

The areas of mesh **implantation** showed no signs of erythema, swelling, exudate, or fragility. Meshes were removed at 7 days, 14 days, and 21 days. . .

DETDESC:

DETD(198)

Parallel studies have been performed in which meshes with dermal and epidermal components were **implanted** into 10 mm.times.10 mm skin biopsies which were then maintained in culture for 14 days and examined histologically. Similar cell. . .

CLAIMS:

CLMS(7)

7. . . . in which the non-biodegradable material is a polyamide, polyester, a polystyrene, a polypropylene, a polyacrylate, a polyvinyl, a polycarbonate, a **polytetrafluoroethylene**, or a nitrocellulose compound.

=> d fro,10

US PAT NO: 4,963,489 :IMAGE AVAILABLE: L3: 10 of 10
DATE ISSUED: Oct. 16, 1990
TITLE: Three-dimensional cell and tissue culture system
INVENTOR: Gail K. Naughton, Groton, VT
Brian A. Naughton, Groton, VT
ASSIGNEE: Marrow-Tech, Inc., La Jolla, CA (U.S. corp.)
APPL-NO: 07/242,096
DATE FILED: Sep. 8, 1988
REL-US-DATA: Continuation-in-part of Ser. No. 38,110, Apr. 14, 1987, which is a continuation-in-part of Ser. No. 36,154, Apr. 3, 1987, Pat. No. 4,721,096, which is a continuation of Ser. No. 853,569, Apr. 18, 1986, abandoned.
INT-CL: :5: C12N 5/00; A01N 1/02
US-CL-ISSUED: 435/240.1, 1, 2, 240.2, 240.23, 240.21; 424/93, 529, 530, 534, 572, 574
US-CL-CURRENT: 435/1.1; 424/529, 530, 534, 572, 574; 435/2, 347, 366, 398, 402
SEARCH-FLD: 435/1, 2, 4, 240.2, 240.243, 240.23, 240.21, 240.1; 424/95, 93
REF-CITED:

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ART-UNIT: 184
PRIM-EXMR: Charles F. Warren
ASST-EXMR: Christopher Low
LEGAL-REP: Pennie & Edmonds

ABSTRACT:
The present invention relates to a three-dimensional cell culture system which can be used to culture a variety of different cells and tissues in vitro for prolonged periods of time. In accordance with the invention, cells derived from a desired tissue are inoculated and grown on a pre-established stromal support matrix. The stromal support matrix comprises stromal cells, such as fibroblasts, grown to subconfluence on a three-dimensional matrix. Stromal cells may also include other cells found in loose connective tissue such as endothelial cells, macrophages/monocytes, adipocytes, pericytes, reticular cells found in bone marrow stroma, etc. The stromal matrix provides the support, growth factors, and regulatory factors necessary to sustain long-term active proliferation of cells in culture. When grown in this three-dimensional system, the proliferating cells mature and segregate properly to form components of adult tissues analogous to counterparts found in vivo.

10 Claims, 10 Drawing Figures

=> d clms,10

US PAT NO: 4,963,489 :IMAGE AVAILABLE: L3: 10 of 10

CLAIMS:

CLMS(1)

What is claimed i[redacted]

1. A living stromal tissue prepared in vitro, comprising stromal cells and connective tissue proteins naturally secreted by the stromal cells attached to and substantially enveloping a framework composed of a biocompatible, non-living material formed into a three dimensional structure having interstitial spaces bridged by the stromal cells.

CLMS(2)

2. The living stromal tissue of claim 1 in which the stromal cells are fibroblasts.

CLMS(3)

3. The living stromal tissue of claim 1 in which the stromal cells are a combination of fibroblasts and endothelial cells, pericytes, macrophages, monocytes, leukocytes, plasma cells, mast cells or adipocytes.

CLMS(4)

4. The living stromal tissue of claim 1 in which the framework is composed of a biodegradable material.

CLMS(5)

5. The living stromal tissue of claim 4 in which the biodegradable material is cotton, polyglycolic acid, cat gut sutures, cellulose, gelatin, or dextran.

CLMS(6)

6. The living stromal tissue of claim 1 in which the framework is composed of a non-biodegradable material.

CLMS(7)

7. The living stromal tissue of claim 6 in which the non-biodegradable material is a polyamide, polyester, a polystyrene, a polypropylene, a polyacrylate, a polyvinyl, a polycarbonate, a **polytetrafluoroethylene**, or a nitrocellulose compound.

CLMS(8)

8. The living stromal tissue of claims 4, 5, 6 or 7 in which the framework is pre-coated with collagen.

CLMS(9)

9. The living stromal tissue of claims 1, 2, 3, 4, 5, 6, or 7 in which the framework is a mesh.

CLMS(10)

10. The living stromal tissue of claim 8 in which the framework is a mesh.